State of Hawaii
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Aquatic Resources
Honolulu, Hawaii 96813

April 28, 2006

Board of Land
and Natural Resources
Honolulu, Hawaii

THE DIVISION OF AQUATIC RESOURCES REQUESTS BOARD OF LAND AND NATURAL RESOURCES (BLNR) AUTHORIZATION/APPROVAL TO ISSUE TWO (2) NORTHWESTERN HAWAIIAN ISLANDS (NWHI) RESEARCH, MONITORING AND EDUCATION PERMITS TO: 1) DR GRETA AEBY, AND 2) DR. STEPHEN KARL, BOTH OF THE HAWAII INSTITUTE OF MARINE BIOLOGY, FOR THE NON-LETHAL SAMPLING OF CORALS FOR INVESTIGATIONS OF CORAL DISEASE AND BLEACHING SUSCEPTIBILITY

Submitted herewith for your authorization and approval is a request for issuance of two (2) NWHI Research, Monitoring and Education permits to Drs. Greta Aeby and Stephen Karl, both of the Hawaii Institute of Marine Biology, University of Hawaii. These permits, described below, will allow activity to occur in the NWHI State marine Refuge (0-3 miles) waters surrounding French Frigate Shoals. The activities covered under this permit will occur from May 18 to June 11, 2006, from the support vessel Hi'ialakai. Ship details are provided with Item F-4.

The proposed activities (below) are consistent with and support the purposes of the Refuge, primarily to better understand and manage the resources within the marine refuge. Understanding the causes of coral disease and developing biological indicators for disease and bleaching are of value to State resource managers. Such information will allow managers to identify specific coral populations that are particularly fragile, or at risk for disease outbreaks or bleaching, and propose appropriate management strategies.

1. RESEARCH, MONITORING AND EDUCATION PERMIT TO AEBY:

Incidences of coral diseases and bleaching have increased dramatically since the 1980's. The Northwestern Hawaiian Islands are considered to be relatively healthy, but they are not immune to the conditions that have led to the decline of other reef systems. In September 2002 the first mass-bleaching event was recorded in the NWHI, and ten coral disease states have now been described from the NWHI. A 2003 outbreak of white syndrome on *Acropora cytherea* at French Frigate Shoals is of particular concern. Aeby is monitoring this population via established permanent transects, and would like to establish 2-4 additional monitoring sites in areas of high *Acropora* cover. This involves the placement of steel pins into dead substrate, tagging of bleached and diseased corals, and photographic documentation. Furthermore, with collaborators, Aeby would like to investigate fish diseases. High levels of infection with bacteria and protozoa have been seen in ta'ape, and a pigmentation disease of kole has been found in the NWHI.

Knowledge of the location and degree of disease outbreaks is important information for resource managers to utilize in decision-making.

2. RESEARCH, MONITORING AND EDUCATION PERMIT TO KARL:

Karl aims to fully characterize the genetic relatedness among individuals of three species of coral on a reef. This information allows him to understand individual variation in disease and bleaching susceptibility. By knowing the genotypes of all individuals on a reef and their current and future disease & bleaching rates, he can provide managers with a predictive tool for monitoring health and differentiating between sensitive and robust sites when making refuge management decisions. Karl will photograph and map the location of all individuals of *Porites lobata* and *Montipora capitata* on a patch reef at FFS, and *Acropora cytherea* at a known disease outbreak location at FFS. He will record the information in a GIS database. He will then take small ($2cm^2$) biopsies, and analyze these at the Hawaii Institute of Marine Biology with microsatellite technologies.

REVIEW PROCESS:

The permits were received by the Division of Aquatic Resources on 1) March 10, 2006 and 2) March 7, 2006. They were sent out for review and comment to the following scientific entities: Division of Aquatic Resources staff (5), Division of Forestry and Wildlife, Northwest Hawaiian Islands Reserve, and the United States Fish and Wildlife Service. Native Hawaiians from the Office of Hawaiian Affairs and Kaho'olawe Island Reserve Commission were also consulted.

Comments received from the Scientific Community (DAR) on Aeby's Permit are summarized as follows:

1) Concern was expressed that some of the species to be sampled are branching forms and provide essential fish habitat

2) Concern was expressed regarding damage caused by transect pin placement, and whether 6 pins were required

3) There was concern that appropriate sampling and diving gear protocols should be developed and enforced so as not to spread any coral disease among sites in the NWHI

4) Concern was expressed about open-ended sampling language (e.g., "targeted species include…") in the original proposal

5) It was questioned whether Aeby could share fish with Bowen (see Item F-6)

Comments received from the Scientific Community (DAR) on Karl's Permit are summarized as follows:

1) Concern was expressed over the large number of samples to be taken

2) There was concern that appropriate sampling and diving gear protocols should be developed and enforced so as not to spread any coral disease among sites in the NWHI

3) It was asked whether Karl's sampling could take place on or near one of Aeby's transects, so as to minimize overall impact at FFS

Comments received from a Native Hawaiian on both the Research, Education and Monitoring Permits are summarized as follows:

1) There was concern for native Hawaiian intellectual property rights for new discoveries and the protection of the resources for their potential product developments.

RESPONSE:

A meeting of DAR staff and HIMB researchers was held on 12 April 2006 to address concerns, and a synopsis of the response to concerns raised is as follows:

Aeby:

1) Utmost care will be taken in sampling branching forms, so as not to damage essential fish habitat.

2) Protocols are already in place for disinfection of sampling and diving gear between sites in the NWHI. All gear is soaked in 10% bleach solution to kill any microorganisms. All samples will be killed by freezing aboard ship.

3) Pins are always placed in dead substrate, not live coral, and utmost care will be taken so as not to damage live coral. Furthermore, it was agreed that the placement of 6 pins in order to assure re-finding the site for future monitoring efforts was preferable to placing fewer pins and possibly not being able to relocate the site.

4) Fish sampling will be limited to the species specified in the permit application, and a maximum of 20 fish of any particular species will be taken. Fish will be shared with Bowen.

5) The Guidelines for Submitting Permit Applications stipulates that, for all permits, the activity must be non-commercial and will not involve the sale of any organism, byproduct, or material collected. Furthermore, the Guidelines state that resources and samples are a public trust, and are not to be used for sale, patent, bioassay, or bio-prospecting, or for obtaining patents or intellectual property rights. This condition will be added to the Permit Terms and Conditions for this, and all future permits. This should address the concerns raised by the Native Hawaiian reviewer.

Karl:

1) Karl pointed out that his small samples are similar to naturally occurring events such as parrotfish feeding. He has chosen species that are abundant across the archipelago, so his impact on a single patch reef at a single site (FFS) is minimal when one looks at the scale of the reef systems in the NWHI.

2) Karl and Aeby agreed that Karl could sample at one of Aeby's sites, to minimize overall impact at FFS

3) Protocols are already in place for disinfection of sampling and diving gear between sites in the NWHI. Gates' samples are killed by freezing aboard the ship. All gear is soaked in 10% bleach solution between sites to kill any microorganisms and eliminate the possibility of disease transmission. All samples will be killed by freezing aboard ship.

4) The Guidelines for Submitting Permit Applications stipulates that, for all permits, the activity must be non-commercial and will not involve the sale of any organism, byproduct, or material collected. Furthermore, the Guidelines state that resources and samples are a public trust, and are not to be used for sale, patent, bioassay, or bio-prospecting, or for obtaining patents or intellectual property rights. This condition will be added to the Permit Terms and Conditions for this, and all future permits. This should address the concerns raised by the Native Hawaiian reviewer.

AMENDMENTS REQUESTED SUBSEQUENT TO APPLICATION SUBMISSION:

Aeby requested via email on April 18, 2006 that Nihoa and Gardner be added to her sampling sites, so that she may sample in the event that coral disease is detected, and that Dr. Jill Zamzow be added to her permit as a sub-permittee.

FINAL STAFF RECOMMENDATIONS:

1) Allow Aeby and Karl to take non-lethal samples of corals, not to exceed the numbers specified in the HIMB Coral Sampling Table (attached). Coral samples are to be taken in coordination with other HIMB personnel: the stated sample sizes and numbers are to be shared by Toonen, Aeby, Karl, Rappé and Gates (see separate permit applications).

2) Allow the placement of 6 steel pins each for the establishment of 2-4 additional *Acropora* transects at FFS, as outlined in the permit application. Allow coral tagging and photography of these transects.

3) Allow mapping and photography of corals as specified in Karl's permit.

4) Allow the take of fishes, up to 20 of each species specified in Aeby's permit.

5) Allow the addition of sites and personnel to Aeby's permit.

RECOMMENDATION:

"That the Board authorize and approve, with stated conditions, 1) a Research, Monitoring and Education Permit to Dr. Greta Aeby of the Hawaii Institute of Marine Biology, and 2) a Research, Monitoring and Education Permit to Dr. Stephen Karl of the Hawaii Institute of Marine Biology, for activities and access within the State waters of the NWHI."

Respectfully submitted,

DAN POLHEMUS
Administrator

APPROVED FOR SUBMITTAL

PETER T. YOUNG
Chairperson

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APPENDIX 1

State of Hawai'i
DLNR
Northwestern Hawaiian Islands State Marine Refuge
Permit Application Form

For Office Use Only
Permit No:
Expiration date:
Date Appl. Received: 3/10/06
Appl. Fee received: N/A
NWHI Permit Review Committee date:
Board Hearing date:
Post to web date:

Type of Permit

☒ I am applying for a **Research, Monitoring & Education** permit. (Complete and mail Application)
- ☐ This application is for a NEW project in the State Marine Refuge.
- ☒ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.

☐ I am applying for a permit for a **Native Hawaiian** permit. (Complete and mail Application)

- ☐ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.

☐ I am applying for a **Special Activity** permit. (Complete and mail Application)

- ☐ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.

Briefly describe **Special permit** activity:

When will the NWHI activity take place?

☒ **Summer** (May-July of 2006 (year)
Note: Permit request must be received before February 1st
Specific dates of expedition 5/23 - 6/16/06

☐ **Fall** (August-November) of ____ (year)
Note: Permit request must be received before May 1st
Specific dates of expedition _____

☐ **Other**

NOTE: INCOMPLETE APPLICATIONS WILL NOT BE ACCEPTED

Please Send Permit Applications to:

NWHI State Marine Refuge Permit Coordinator
State of Hawai'i
Department of Land and Natural Resources
Division of Aquatic Resources
1151 Punchbowl Street, Room 330
Honolulu, Hawai'i 96813

NWHI State Marine Refuge Permit Application
See Appendix 2 for Application Instructions

Section A – Applicant Information	
1. Project Leader (attach Project Leader's CV or resume) ☒ CV attached Aeby, Greta Name: Last, First, Middle Initial	Title Asst. Researcher
2. Mailing Address (Street/PO Box, City, State, Zip) PO Box 1346 Kaneohe, HI 96744	Telephone (808) 236-7400 Fax (808) 236-7443 Email Address Greta@hawaii.edu
3. Affiliation (Institution/Agency/Organization) HIMB	For graduate students, Major Professor 's Name & Telephone
4. Sub-Permittee/Assistant Names, Affiliations, and Contact Information ☐ CV or resume attached	
5. Project Title Investigation of fish & coral disease in the NWHI	
6. Applicant Signature	7. Date (mm/dd/yyyy) 3/9/06

Section B: Project Information
8. **(a) Project Location** ☒ NWHI State Marine Refuge (0-3 miles) waters surrounding: ☐ Nihoa Island ☐ Necker Island (Mokumanamana) ☒ French Frigate Shoals ☐ Laysan ☐ Maro ☐ Gardner Pinnacles ☐ Lisianski Island, Neva Shoal ☐ Pearl and Hermes Atoll ☐ Kure Atoll, State Wildlife Refuge ☐ Other NWHI location Describe project location (include names, GPS coordinates, habitats, depths and attach maps, etc. as appropriate).

NWHI State Marine Refuge Permit Application

Section A - Applicant Information

1. Project leader: Dr. Greta Aeby
Assistant researcher

2. PO Box 1346
Kaneohe, HI 96744
Phone: 808-236-7437
FAX: 808-236-7443
Email: greta@hawaii.edu

3. Hawaii Institute of Marine Biology
4. Assistants: Dr. Thierry Work, USGS, Thierry_work@usgs.gov. Up to three other assistants will be named at a later date.

5. Project title: Investigation of fish and coral disease in the NWHI

Section B – Project Information

8a. Project location: Surveys will be conducted at 5 permanent monitoring sites established in 2005 at FFS (Table 1). If time allows, we would like to set up an additional 2-4 sites in areas of high *Acropora* cover.

Table 1. Permanent sites for disease monitoring set up in 2005

Island	Site	Type	Depth (ft)	Lat	Long
FFS	tc12	lagoon	37	23 38.278	166 10.779
	tc21	forereef	37	23 50.812	166 19.629
	tc30	lagoon	18	23 50.982	166 17.827
	r11	lagoon	80	23 38.149	166 11.138
	r16	shelf	30	23 51.011	166 19.746

8c. Collection of specimens

For coral reproduction studies would like to sample 8 colonies of *Acropora cytherea* with each disease (16 colonies total) as well as 8 control colonies. Two small (3-5cm) samples will be taken per diseased colony and one sample per control colony. Total at FFS =24 samples. For microbiological culture of *Acropora* white syndrome we would like to take one sample (3-5 cm) per infected colony with a maximum of 10 colonies sampled. Total = 10 samples. Samples from other diseased corals will be collected as encountered. . For fish disease studies, targeted species include taape (*Lutjanus kasmira*), the yellowfin goatfish (*Mulloidichthys vanicolensis*), the yellowstripe goatfish (*M. flavolineatus*),

(b) check all actions to be authorized:

☒ Enter the NWHI Marine Refuge waters

☒ Take (harvest) ☐ Possess ☐ Transport (☐ Inter-island ☐ Out-of-state)

☐ Catch ☐ Kill ☐ Disturb ☐ Observe

☐ Anchor ☐ Land (go ashore) ☐ Archaeological research

☐ Interactions with Sea Turtles or Monk Seals ☐ Interactions with Seabirds

☐ Interactions with Live Coral, Ark Shells or Pearl Oysters

☐ Interactions with Jacks, Grouper or Sharks

☐ Conduct Native Hawaiian religious and/or cultural activities

☐ Other activities _____

(c) Collection of specimens – collecting activities (would apply to any activity):

Organisms or objects (List of species, if applicable, add additional sheets if necessary):

Common name	**Scientific name**	**No. & size of specimens**	**Collection Location(s)**

(d) What will be done with the specimens after the project has ended?

(e) Will the organisms be kept alive after collection? ☐ yes ☒ no

- Specific site/location _____
- Is it an open or closed system? ☐ open ☐ closed
- Is there an outfall? ☐ yes ☐ no
- Will these organisms be housed with other organisms? If so, what are the other organisms?

manybar goatfish (*Parupeneus multifasciatus*), sidespot goatfish (*P. pleurostigma*), Pfluger's goatfish (*M. pflugeri*) and kole (*Ctenochaetus strigosus*). A maximum of 20 fish per species would be collected.

8d. Samples will be used for histology, reproductive studies, microbiology and molecular studies which will use up all samples.

9. Purpose of activity

Background

Global climate change and human activities are placing coral reef ecosystems at risk. Coral reefs worldwide are now declining at an alarming rate. Mass bleaching events have increased dramatically since the 1980's and have usually been linked to El Nino or global warming-related increases in annual sea surface temperature (Brown 1997, Barber et al. 2001). The El Nino Southern Oscillation (ENSO) conditions during 1997 to 1998 resulted in worldwide bleaching from the Western Atlantic to the Great Barrier Reef. ENSO events have increased in frequency and duration in the past two decades (Barber et al. 2001, Walker 2001) and it has been predicted that the frequency and severity of coral bleaching will also continue to rise (Hoegh-Guldberg 1999).

The incidence of coral diseases has also greatly increased (Santavy and Peters 1997, Green and Bruckner 2000). In the Florida Keys, Porter et al. (2001) report that between 1996 and 1998 the number of survey stations containing diseased individuals increased by 265%. They also report dramatic increases in the number of different coral species exhibiting disease. Coral disease has also been reported to be responsible for the dramatic decline of Acroporids, one of the major frame-building corals in the Florida Keys, changing the structure and function of the coral reef ecosystem (Aronson & Precht 2001). Despite the major impact disease can have on reef systems, the etiology of most coral diseases remains unclear (Santavy and Peters 1997, Richardson 1998). The causative agents, mechanism of pathogenesis and link to environmental or anthropogenic stress are still largely unknown (Richardson 1998, Green & Bruckner 2000).

The reefs of the Northwestern Hawaiian Islands (NWHI) are considered to be relatively healthy but they are not immune to the conditions that have led to the decline of other reef systems. In September 2002 the first mass-bleaching event was recorded on the reefs of the NWHI. In the three northwestern most atolls of the Archipelago (Pearl & Hermes, Midway and Kure) over half of all sites had significant bleaching (Aeby et al. 2003, Kenyon et al., in press). Ten coral disease states have now been described from the NWHI (Aeby in press$_a$). An outbreak of white syndrome on *Acropora cytherea* was found at French Frigate Shoals (FFS) in 2003 and appears to be spreading throughout FFS (Aeby, 2006). *Acropora* white syndrome has resulted in significant reduction of acroporid corals in some areas of the Marshall Islands (Jacobson 2006). It is important for management agencies to have a through understanding of the vulnerability of these reefs to the different threats in order to develop appropriate management plans for their conservation.

We are proposing to re-survey permanent sites established at FFS in 2005 and if time permits establish 2-4 additional sites in areas of high *Acropora* cover. *Acropora* white syndrome was discovered at FFS in 2003 (Aeby 2006) and is a disease of concern

in the NWHI. Establishment of permanent sites will allow us to determine both temporal and spatial changes in diseases through time and to determine the ultimate affect of disease on the health of the ecosystem. We will measure changes in disease levels through time, rates of tissue loss from different diseases, patterns of disease transmission among colonies, rate of spread of disease and evaluate changes in coral cover and coral species composition. This information can only be acquired through re-surveying the same transects on a reef and thus requires placement of permanent markers. From these surveys we will also be able to collect small samples of diseased and healthy coral for follow up laboratory investigations. The laboratory investigations will allow us to develop the tools necessary to elucidate the etiology, pathophysiology, and epizootiology of disease in the major groups of scleractinian corals in Hawaii. We will have an onboard microbiology lab allowing a through investigation of the microbiota of encountered coral diseases.

Disease can affect coral communities directly through mortality of colonies (partial or whole) resulting in reduced coral cover or indirectly through sub-lethal events such as reduced growth, resilience or reproduction. *Acropora cytherea* at FFS is affected by two diseases (*Acropora* white syndrome and *Acropora* growth anomalies) which are of concern to managers. Permanent sites have been set up at FFS to understand the progression of these two coral diseases and we would now like to examine the affect of these two diseases on the reproductive output of *Acropora cytherea*. *A. cytherea* is know to reproduce during May/June in the NWHI (Kenyon 1992) and so the timing of this cruise will allow us to compare the reproductive output of infected versus uninfected colonies.

Diseases in marine ecosystems are not only limited to corals. Fibropapillomatosis of green turtles has been known in Hawaii since the 1950s (Balaz 1991). More recently, high levels of infections with bacteria and protozoa have been seen in taape (*Lutjanus kasmira*) (Work et al. 2003). Taape were introduced into Hawaii in the 1950s (Randall 1987) and have spread all the way to Midway Atoll. Taape are closely associated with certain native fish such as goatfish (*Mulloidichthys* sp.) (Friedlander et al. 2002) and goatfish from the main Hawaiian Islands have been found to be infected with some of the same diseases as taape (Work et al. unpub. data). We would like to examine whether these diseases have spread up into the NWHI. In addition, in May 2005 a pigmentation disease of kole (*Ctenochaetus strigosus*) was found in the NWHI. These fish have obvious altered skin pigmentation and a reduced body condition. We would like to do further studies on any infected fish encountered.

Objectives:

1) To re-survey permanent sites established in 2005 for the assessment of disease dynamics.
2) To establish 2-4 more permanent sites in areas of high *Acropora* cover.
3) Culture microbiota associated with *Acropora* white syndrome.
4) Examine the reproductive cost of *Acropora* white syndrome and growth anomalies.
5) Examine taape and native goatfishes from NWHI for presence of disease.
6) Examine any kole (*Ctenochaetus strigosa*) affected by pigmentation disease.

Justification:

Recently, outbreaks of novel diseases have occurred in the world's oceans. Mass mortality of sea fans occurred in reefs of the Caribbean and Florida Keys. The pathogen, *Aspergillus*, was thought to be a new species that originated from terrestrial sources (Smith et al., 1996, Rosenberg and Ben-Haim 2002). White pox, a lethal disease of *Acropora palmata,* was first documented on the reefs in 1996 and was found to be caused by a common fecael enterobacterium found in the human gut (Patterson et al. 2002). Current models of global climate change predict a significant increase in sea surface temperature (Kleypas et al. 1999, Walker 2001). Elevated temperatures have been shown to accelerate the growth rate and pathogenecity of pathogens (Porter et al., 2001) and so it has been predicted that coral disease will become even more common and widespread (Porter et al. 2001, Rosenberg and Ben-Haim 2002). Disease in the Indo-Pacific is on the rise (Willis et al. 2005, Jacobson 2006) and so it is important we gain a better understanding of the disease dynamics on the reefs of the NWHI. This knowledge will be critical if we are to effectively address coral disease outbreaks and provide appropriate management recommendations to resource biologists. This study will focus on *Acropora* which does not occur in the main Hawaiian Islands.

10. Procedure:

Techniques:

Surveys: Re-survey of established sites will follow established protocol. Two 25 m lines will be laid out. A diver will then swim over the lines during which all corals within one half meter of either side of the transect lines will be identified to specie, counted, and assigned to a size class (0-5cm; 6-10cm; 11-20cm; 21-40cm; 41-80cm; 81-150cm; >150cm.). In the same manner, a second diver will swim over the lines and examine all corals for signs of bleaching or disease. Bleached colonies will be assigned a bleaching category: 0-no bleaching; 1- 10-30%; 2-30-50%; 3-50-100%; 4- 100%; 5-mortality. For corals exhibiting disease, a general description of the condition will be recorded, the coral will be photographed and a specimen will be collected for histopathological and molecular examination. All enumerated bleached and diseased corals will also be assigned a size class consistent with the population counts. Individual colonies tagged in 2005 will be relocated, remarked and photographed. Any new infected colonies found along the transect will be photographed and tagged.

To establish new permanent sites, two 25-meter lines will be marked with steel pins at 5-meter intervals. GPS units will be recorded at the start of each transect. This method has been used successfully by USFWS personnel at sites within the NWHI. Both transect lines will be surveyed for coral disease and diseased colonies encountered along the belt transect will be marked and their position recorded.

***Coral reproduction*:** Small samples from infected and non-infected *Acropora cytherea* will be collected, preserved in 10% formalin and decalcified. For each sample piece, 5 fertile polyps from a 1 cm length of tissue behind the sterile growing tip will be randomly chosen for dissection. Maximum and median diameter of each oocyte will be measured and oocyte size computed as a geometric mean. The length of each individual testis in each polyp will also be recorded. Histological sections of polyps, prepared by standard techniques will be examined to determine maturity of gonads.

Bacterial Isolation: Water samples and mucus from diseased corals will be plated onto marine agar (MA) and a *Vibrio* selective media (TCBS; thiosulfate citrate bile salt sucrose) (Difco-BBL, Franklin Lakes NJ) for grow out of culturable bacteria. In brief, fifty µL of sample will be inoculated onto each media culture plate and spread using a heat-sterilized glass spreader. Cultures will be incubated at room temperature until colonies are visible (1-3 days on average) Bacterial colonies will be collected from the MA and TCBS plates using a variety of selection criteria (colony elevation, color, shape, margin, motility, and surface texture). All unique colonies will be archived as glycerol stocks and frozen at -80°C.

Fish: Fish will be weighed and measured (standard and fork length), examined systematically externally and internally, and gross lesions documented. For histopathology, sections of skeletal muscle, skin, spleen, liver, cranial and caudal kidneys, swim bladder, brain, heart, gill, and gonad, small intestines, and stomach will be excised and fixed in 10% neutral buffered formalin. Tissues will sectioned, dehydrated in alcohol series, embedded in paraffin, sectioned at 5 µm, placed on microscope slides, stained with hematoxylin and eosin, and examined using a light microscope. Special stains will be used as appropriate to identify fungi, bacteria, or protozoa. Histopathology will allow us to characterize microscopic morphology of disease, will provide systematic evaluation of cellular changes that occur in disease, and will afford the opportunity to detect microorganisms and the host response to these organisms. Targeted fish species include taape (*Lutjanus kasmira*), kole (*Ctenochaetus strigosus*), the yellowfin goatfish (*Mulloidichthys vanicolensis*), the yellowstripe goatfish (*M. flavolineatus*), manybar goatfish (*Parupeneus multifasciatus*), sidespot goatfish (*P. pleurostigma*), and Pfluger's goatfish (*M. pflugeri*). A maximum of 20 fish per species would be collected.

11. Funding source. The project will be funded by the NWHICRER-HIMB partnership NMSPO MOU 2005-008/66882.

12. Literature cited

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13. What types of insurance do you have in place?

NOAA Ship HI`IALAKAI is a U.S. Government-owned and –operated research vessel and is self-insured by the U.S. Government.

14. What certifications/inspections do you have scheduled for your vessel?

- Rat Free (scheduled with U.S. Dept. of Health and Human Services for April 2006)
- Hull Inspection (scheduled with Hawaii Institute of Marine Biology biologists (normally Scott Godwin) prior to projects working in the Northwestern Hawaiian Islands (NWHI)) to ensure no nuisance algae or other fouling species are transported to the NWHI.
- Ballast water information is transmitted to USCG as required by CFR Title 33, Vol. 2, Parts 151.1500 to 199; IMO Resolution A.868(20); and USCG COMDTPUB P16700.4

15. Permits: The proposed research is subject to the jurisdiction of the U.S. Fish & Wildlife Service and its Special Use Permit requirements, NWHI Coral Reef Ecosystem Reserve and to State of Hawaii jurisdiction and approvals. Applications to the State of Hawaii, FWS and the NWHI CRER have been submitted.

16. This research will be conducted in collaboration with ongoing annual disease monitoring conducted during NWHI RAMPs. All permanent sites established will be reported to the US FWS coral biologist for possible inclusion into their monitoring program.

Section C. Logistics

17. The cruise is scheduled for May 23-June16. Dates within the NWHI State marine refuge are scheduled for May 24-June 3, 2006. Hiialakai is scheduled to be in the NWHI at the following places and dates contingent upon weather. Hono-Nihoa 5/23-24; Nihoa; 5/25-27; FFS 5/28-6/1; Gardner 6/2-6/3/06.

18. Gear and materials

Dive gear
Collecting equipment
Chemicals (formalin, z-fix)

19. Fixed installations

Transect markers: steel pins
Colony markers; cable ties

20. Time line

Fall 2006: histology processing, coral reproduction dissections. Spring 2007: data analysis and report writing

21. Vessel Information

Vessel Name – NOAA Ship HI`IALAKAI
IMO Number – 8835619
Vessel Owner – U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration (NOAA)
Flag – USA
Captain's Name – CDR Scott Kuester, NOAA
Chief Scientist or Project Leader – Randall Kosaki, Ph.D., NOAA
Vessel Type – Oceanographic Research
Call Sign – WTEY
Length – 224 feet
Gross Tonnage – 1,914
Port of Embarkation – Honolulu
Last port vessel will have been at prior to this embarkation – Pago Pago, Amer. Samoa
Total Ballast Water Capacity:
Volume – 487 m3 (128,834 U.S. gal.)
Total number of ballast tanks on ship – 10
Total Fuel Capacity:
228,642 U.S. gal. at 98% capacity
Total number of fuel tanks on ship – 15
Other fuel/chemicals to be carried on board and amounts: gasoline – as much as 700 U.S. gal.; lube oil – as much as 10,442 U.S. gal.; numerous other industrial and household chemicals used to operate a 224-foot research vessel
Number of tenders/skiffs aboard and specific type of motors:

Ship's own tenders - 1 each 10 m AMBAR Marine jet boat with Yanmar 370-hp, Diesel inboard engine
1 each 8 m AMBAR Marine jet boat with Yanmar 315-hp, Diesel inboard engine
2 each 17.5 ft Zodiac inflatable boats, each with one Honda 50-hp, 4-stroke, outboard gasoline engine
1 each 19 ft AMBAR Marine rescue boat with Honda 115-hp, 4-stroke, outboard gasoline engine

Program-provided tenders – 19' Boston Whaler with 135 hp Honda four-stroke outboard

Does the vessel have the capability to hold sewage and grey-water? Describe in detail.
The ship has a 4,000 U.S. gal Collection Holding Tank for sewage and grey water. In those waters where effluent may NOT be discharged, sewage and grey water are held in this tank until the ship is in waters where sewage and grey water may be discharged. The ship has a U.S. Coast Guard-approved Marine Sanitation Device (Omnipure model MSD 12 MC) which is used to treat sewage and grey water in those waters where effluent may be discharged.

Does the vessel have a night-time light protocol for use in the NWHI? Describe in detail. Navigation lights are on 24-hours/day. Work lights are put on at night only when conducting CTD operations. Weather decks are not illuminated at night.

On what workboats (tenders) will personnel, gear and materials be transported within the State Marine Refuge? - Personnel, gear and materials may be transported within the State Marine Refuge by the ship or any of the 5 ship's small boats listed above or by the program-provided small boat listed above.

How will personnel, gear and materials be transported between ship and shore? – Personnel, gear and materials may be transported between ship and shore by any of the 5 ship's small boats listed above or by the program-provided small boat listed above.

If applicable, how will personnel be transported between islands within any one atoll? - Personnel may be transported between islands within any one atoll by any of the 5 ship's small boats listed above or by the program-provided small boat listed above.

HIMB Coral Sampling Table

Coral species	Site	number	size
Acropora cytherea	FFS	50	6 cm square
Acropora cytherea	FFS	450	2 cm square
Acropora cytherea	Nihoa	50	6 cm square
Acropora cytherea	Gardner	50	6 cm square
Acropora nasuta	FFS	5	2 cm square
Acropora nasuta	Nihoa	5	2 cm square
Acropora nasuta	Gardner	5	2 cm square
Acropora paniculata	FFS	5	2 cm square
Acropora paniculata	Nihoa	5	2 cm square
Acropora paniculata	Gardner	5	2 cm square
Pocillopora damicornis	FFS	5	2 cm square
Pocillopora damicornis	Nihoa	5	2 cm square
Pocillopora damicornis	Gardner	5	2 cm square
Pocillopora meandrina	FFS	50	6 cm square
Pocillopora meandrina	Nihoa	50	6 cm square
Pocillopora meandrina	Gardner	50	6 cm square
Pocillopora eydouxi	FFS	5	2 cm square
Pocillopora eydouxi	Nihoa	5	2 cm square
Pocillopora eydouxi	Gardner	5	2 cm square
Porites lobata	FFS	450	2 cm square
Porites lobata	FFS	50	6 cm square
Porites lobata	Nihoa	50	6 cm square
Porites lobata	Gardner	50	6 cm square
Porites brighami	FFS	5	2 cm square
Porites brighami	Nihoa	5	2 cm square
Porites brighami	Gardner	5	2 cm square
Porites lichen	FFS	5	2 cm square
Porites lichen	Nihoa	5	2 cm square
Porites lichen	Gardner	5	2 cm square
Montipora capitata	FFS	450	2 cm square
Montipora capitata	FFS	50	6 cm square
Montipora capitata	Nihoa	50	6 cm square
Montipora capitata	Gardner	50	6 cm square
Montipora patula	FFS	5	2 cm square
Montipora patula	Nihoa	5	2 cm square
Montipora patula	Gardner	5	2 cm square
Leptastrea bewickensis	FFS	5	2 cm square
Leptastrea bewickensis	Nihoa	5	2 cm square
Leptastrea bewickensis	Gardner	5	2 cm square
Pavona varians	FFS	50	6 cm square
Pavona varians	Nihoa	50	6 cm square
Pavona varians	Gardner	50	6 cm square
Fungia scutaria	FFS	50	6 cm square
Fungia scutaria	Nihoa	50	6 cm square
Fungia scutaria	Gardner	50	6 cm square
Tubastraea coccinea	FFS	50	2 cm square
Tubastraea coccinea	Nihoa	50	2 cm square
Tubastraea coccinea	Gardner	50	2 cm square

* if diseased corals are encountered for any species: If sample size is less than 30, sample size will be increased to 30 (10 each site) and if chip size is 2 cm^2, chip size will be increased to 6 cm^2.

Coral collectors will be working in close coordination with one another. One team will be collecting larger (6 cm) samples, the other team will collect small (2 cm) samples. They will communicate to ensure that oversampling does not occur.

Greta Smith Aeby

EDUCATION

2000-2002 **Post-doctoral research associate,** University of West Florida
Field of study: coral bleaching and disease

1998 **Ph.D., University of Hawaii at Manoa**
Field of study: Coral biology, coral reef ecology, evolution and ecology of a marine trematode parasite of corals and reef fish.

1981 **B.S., University of New Mexico**
Major: Biology Minor: Chemistry

EMPLOYMENT

Research:

9/05- Assistant researcher, Hawaii Institute of Marine Biology.
Research on the epizootiology of coral disease.

9/02- 9/05 NWHI Research Coordinator, Hawaii Dept. of Land and Nat. Resources
Assessment of the frequency of coral bleaching and disease on reefs in the Northwestern Hawaiian Islands. Designing a management plan for and coordinating research projects occurring in the NWHI.

7/00-8/02 Post-doctoral research associate, University of West Florida
Factors affecting the susceptibility of corals to bleaching. The role of reef fish in the susceptibility and spread of black band disease of corals.

1/98-6/00 Researcher, Hawaii Cooperative Fishery Research Unit
Fisheries ecology of deep-water bottomfish. Trophic interactions between introduced blueline snapper and native deep-water snappers. Pisces V submersible deep-water habitat surveys.

1990-1992 Research Assistant, Hawaii Institute of Marine Biology
Population biology of selected mobile finfish species within Kaneohe Bay.

1995-1997 Research Assistant, Hawaii Institute of Marine Biology
Research assistant. Coordinated SCUBA diving at HIMB including maintenance of compressors and tanks.

PUBLICATIONS

Aeby, G.S. 2006. Outbreak of coral disease in the Northwestern Hawaiian Islands. Coral Reefs 24(3):481.

Aeby, G.S. In press. Baseline levels of coral disease in the Northwestern Hawaiian Islands. Proc. 3rd. Symp. Northwestern Hawaiian Islands.

Kenyon, J. G. Aeby, R. Brainard, J. Chojnacki, M. Dunlap, C. Wilkinson. In press. Mass coral bleaching on high-latitude reefs in the Hawaiian Archipelago. Proc. 10th Int. Coral Reef Symp.

Aeby, G.S. and Santavy, D.L. In press. Factors affecting the susceptibility of the coral *Montastrea faveolata* to black-band disease. Mar Ecol Prog Ser

Kenyon, J., Vroom, P., Page, K., Dunlap, M., Wilkinson, C. and G. Aeby. 2006. Community Structure of Hermatypic Corals at French Frigate Shoals, Northwestern Hawaiian Islands: Capacity for Resistance and Resilience to Selective Stressors. Pac Sci 60(2):153-175.

Work, T. and G. Aeby. In press. Systematically describing gross lesions in corals. Dis Aquatic Org

Maragos, J., G. Aeby, D. Gulko, J. Kenyon, D. Potts, D. Siciliano, and D. VanRavensway. 2004. The 2000-2002 Rapid Ecological Assessment of Corals in the Northwestern Hawaiian Islands, Part I: Species and Distribution. Pacific Science 58(2):211-230 .

Aeby, G. 2003. Corals in the genus *Porites* are susceptible to infection by a larval trematode. Coral Reefs 22:216.

Aeby, G.S., Kenyon, J., Maragos, J. and Potts, D. 2003. First record of mass coral bleaching in the Northwestern Hawaiian Islands. Coral Reefs 22:256.

Aeby, G.S. 2002. Trade-offs for the butterflyfish, *Chaetodon multicinctus*, when feeding on coral prey infected with trematode metacercariae. Behav. Ecol. Sociobiol. 52:158-163.

Aeby, G.S. 1998. A digenean metacercaria from the reef coral, *Porites compressa*, experimentally identified as *Podocotyloides stenometra*. J. Parasitol. 84:1259-1261.

Clarke, T. and Aeby, G.S. 1998. The use of small mid-water attraction devices for the investigation of the pelagic juveniles of carangid fishes in Kaneohe Bay, HI. Bull. Mar. Sci. 62:947-955.

Gochfeld, D.J. and Aeby, G.S. 1997. Control of populations of the coral-feeding nudibranch, *Phestilla sibogae*, by fish and crustacean predators. Mar. Bio. 130:63-69.

Aeby, G.S. 1993. The potential effect the ability of a coral intermediate host to regenerate may have had on the evolution of its association with a marine parasite. Proc. Seventh Intl. Coral Reef Symp. 2:809-815.

Aeby, G.S. 1991. Behavioral and ecological relationships between a parasite and its hosts. Pacific Science 45:263-269.

GRANTS

2005 *Investigation of coral disease on the reefs of American Samoa.* (G. Aeby, T. Work, D. Fenner). $13,981. American Samoa Coral Reef Advisory Group.

2005 *Chemical mechanisms of disease resistance in Hawaiian corals.* (D. Gochfeld, G. Aeby, J. Miller). $26,204. Hawaii Coral Reef Initiative.

2004 *Coral Disease on the reefs of American Samoa.* (G. Aeby, T. Work, E. Didonato). $12,800. American Samoa Coral Reef Advisory Group.

2004 *Investigation of disease in coral and reef fish on Maui.* (G. Aeby, J. Parrish, T. Lewis, T. Work, S. Coles, J. Casey). $79,900. Hawaii Coral Reef Initiative.

2004 *Anthropogenic organic contaminants on coral reefs: global atmospheric deposition or local sources?*(G. Garrison, G. Aeby, B. Walsh, C. Orazio, J.Carroll). $49,500. USGS State Partnership Program.

2003 *Investigation of disease in introduced and native Hawaiian fish.* (M. Kent, T. Work, G. Aeby, W. Font). $69,000. Hawaii Coral Reef Initiative.

2003 *Investigation of coral disease on the reefs of Oahu.* (T. Lewis, G. Aeby, T. Work, S. Coles). $79,000. Hawaii Coral Reef Initiative.

2003 Edwin W. Pauley 2003 Summer Program – *Pacific Coral Health Workshop and Molecular Biology Techniques* (P. Jokiel, G. Aeby, E. Cox, J. Leong, T. Lewis). $75,000, funded by the Pauley Foundation.

THIERRY MARTIN WORK

PO Box 2302
Honolulu, HI 96804
(808) 247-6989

USGS-NWHC-HFS
PO Box 50167
Honolulu, HI 96850
(808) 541-3445

EDUCATION

1988-91	• Residency in Wildlife medicine, University of California, Davis (UCD).
1990	• Master of Preventive Veterinary Medicine (UCD).
1988	• Doctor of Veterinary Medicine (UCD).
1986	• Master of Science, Entomology (UCD).
1983	• Bachelor of Science *cum laude*, Entomology, Texas A&M University.

CURRENT EMPLOYMENT

Project leader of the Honolulu Field Station (HFS) for the US Geological Survey National Wildlife Health Center (USGS-NWHC) which required developing client contacts, and hiring and managing a technician and volunteers. The HFS, is the primary resource for wildlife health related issues in Hawaii and US territories in the Pacific Basin. The HFS serves or coordinates with a wide variety of public agencies and private organizations. HFS conducts research and field work on diseases of free-ranging terrestrial and marine wildlife.

EMPLOYMENT HISTORY

1992-	• Wildlife Disease Specialist for USGS-NWHC-HFS.
1988-91	• Wildlife Veterinarian, Wildlife Investigations Laboratory, CDFG.

LICENSES AND PROFESSIONAL ASSOCIATIONS

1992-	• American Association of Zoo Veterinarians
1991-	• American Association of Wildlife Veterinarians
1989-	• Wildlife Disease Association
1988-	• Licensed and accredited veterinarian in California
	• American Veterinary Medical Association

HONORS

1996	• National Biological Service STAR award
1994	• National Biological Survey Quality Performance Award
1993	• US Fish and Wildlife Service Special Achievement Award
1983	• Phi Kappa Phi; Phi Sigma
1981-83	• Distinguished Student (honor roll)
2004	• Sigma Xi

RECENT PEER-REVIEWED PUBLICATIONS

Work, T. M., G. H. Balazs, R. A. Rameyer, R. M. Morris. 2004. Retrospective pathology survey of green turtles (*Chelonia mydas*) with fibropapillomatosis in the Hawaiian Islands, 1993-2003. Diseases of Aquatic Organisms 62:163-176.

Greenblatt, R. J., S. L. Quackenbush, R. N. Casey, R. Rovnak, G. H. Balazs, **T. M. Work**, J. W. Casey, and C. A. Sutton. 2005. Genomic variation of the fibropapilloma-associated marine turtle herpesvirus across seven geographic areas and three host species. Journal of Virology 79:1125-1132.

Work, T. M. 2005. Cancer in sea turtles. Hawaii Medical Journal 64:23-24.

Work, T. M., G. H. Balazs, J. L. Schumacher and A. Marie. 2005. Epizootiology of spirorchiid infection in green turtles (*Chelonia mydas*) in Hawaii. Journal of Parasitology. 91:871-876.

Work, T. M. and R. A. Rameyer. 2005. Evaluating coral reef health in American Samoa. Coral Reefs 24:384-390.

Roffe, T. J. and **T. M. Work**. 2005. Wildlife health and disease investigations. *In.* Braun C. E. (ed.) Techniques for wildlife investigations and management, 6th edition. The Wildlife Society, Bethesda, MD. pp. 616-631.

Yabsley, M. J., **T. M. Work**, and R. A. Rameyer 2005. Molecular phylogeny of *Babesia poelea* from brown boobies (*Sula leucogaster*) from Johnston Atoll, Central Pacific. Journal of Parasitology (in press)

Greenblatt, R. J., **T. M. Work**, P. Dutton, C. A. Sutton, T. R. Spraker, R. N. Casey. C. E. Diez, D. Parker, J. St. Ledger, G. H. Balazs, and J. W. Casey. 2005. Geographic variation in marine turtle fibropapillomatosis. Journal of Zoo and Wildlife Medicine 36:527-530.

Reynolds, M.,**T. M. Work** . 2005 Mortality in the endangered Laysan Teal *Anas laysanensis*: conservation implications. Wildfowl 55:31-48.

Work, T. M., and G. S. Aeby. 2006. Systematically describing gross lesions in corals. Diseases of Aquatic Organisms (in press)

LANGUAGES

French, Spanish [fluent]; Russian, Tok Pisin [working knowledge].

APPENDIX 1

State of Hawai'i
DLNR
Northwestern Hawaiian Islands State Marine Refuge
Permit Application Form

For Office Use Only
Permit No:
Expiration date:
Date Appl. Received: N/A
Appl. Fee received: 3/7/06
NWHI Permit Review Committee date:
Board Hearing date:
Post to web date:

Type of Permit

☑ I am applying for a **Research, Monitoring & Education** permit. (Complete and mail Application)
- ☑ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.

☐ I am applying for a permit for a **Native Hawaiian** permit. (Complete and mail Application)

- ☐ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.

☐ I am applying for a **Special Activity** permit. (Complete and mail Application)

- ☐ This application is for a NEW project in the State Marine Refuge.
- ☐ This application is for an ANNUAL RENEWAL of a previously permitted project in the State Marine Refuge.

Briefly describe **Special permit** activity:

When will the NWHI activity take place?

☑ **Summer** (May-July of 2006 (year)
Note: Permit request must be received before February 1st
Specific dates of expedition 18 May - 14 June

☐ **Fall** (August-November) of ____ (year)
Note: Permit request must be received before May 1st
Specific dates of expedition _____

☐ **Other**

NOTE: INCOMPLETE APPLICATIONS WILL NOT BE ACCEPTED

Please Send Permit Applications to:

NWHI State Marine Refuge Permit Coordinator
State of Hawai'i
Department of Land and Natural Resources
Division of Aquatic Resources
1151 Punchbowl Street, Room 330
Honolulu, Hawai'i 96813

NWHI State Marine Refuge Permit Application
See Appendix 2 for Application Instructions

Section A – Applicant Information

1. Project Leader (attach Project Leader's CV or resume) ☑ CV attached Karl, Stephen A. Name: Last, First, Middle Initial	Associate Researcher Title
2. Mailing Address (Street/PO Box, City, State, Zip) HIMB P.O. Box 1346 Kaneohe, HI 96744	Telephone (808) 236-7478 Fax (808) 236-7443 Email Address skarl@hawaii.edu
3. Affiliation (Institution/Agency/Organization) HIMB, University of Hawaii	For graduate students, Major Professor 's Name & Telephone N/A
4. Sub-Permittee/Assistant Names, Affiliations, and Contact Information ☐ CV or resume attached None	
5. Project Title Micro-spatial scale assessment of the genetic architecture of coral reefs	
6. Applicant Signature Stephen A. Karl	7. Date (mm/dd/yyyy) 03/01/2006

Section B: Project Information

8. **(a) Project Location**

☑ NWHI State Marine Refuge (0-3 miles) waters surrounding:

- ☐ Nihoa Island
- ☐ Necker Island (Mokumanamana)
- ☑ French Frigate Shoals
- ☐ Laysan
- ☐ Maro
- ☐ Gardner Pinnacles
- ☐ Lisianski Island, Neva Shoal
- ☐ Pearl and Hermes Atoll
- ☐ Kure Atoll, State Wildlife Refuge
- ☐ Other NWHI location

Describe project location (include names, GPS coordinates, habitats, depths and attach maps, etc. as appropriate).

Gin Island, French Frigate Shoals: 23° 43'51.9" N, 166· 09'34.67" W, 0 - 20 meters

(b) check all actions to be authorized:

☑ Enter the NWHI Marine Refuge waters

☑ Take (harvest) ☑ Possess ☑ Transport (☑ Inter-island ☐ Out-of-state)

☐ Catch ☐ Kill ☑ Disturb ☐ Observe

☐ Anchor ☐ Land (go ashore) ☐ Archaeological research

☐ Interactions with Sea Turtles or Monk Seals ☐ Interactions with Seabirds

☑ Interactions with Live Coral, Ark Shells or Pearl Oysters

☐ Interactions with Jacks, Grouper or Sharks

☐ Conduct Native Hawaiian religious and/or cultural activities

☐ Other activities _____

(c) Collection of specimens – collecting activities (would apply to any activity):

Organisms or objects (List of species, if applicable, add additional sheets if necessary):

Common name	Scientific name	No. & size of specimens	Collection Location(s)
Lobe Coral	Porites lobata	500 - 3 cm^2**	Patch reef on FFS
Rice Coral	Montipora capitata	500 - 3 cm^2**	Patch reef on FFS

** - If this application is approved, a subset of these will be shared with Dr. Ruth Gates who is submitting a separate application for the same cruse.

(d) What will be done with the specimens after the project has ended?

DNA will be extracted and archived at HIMB. Samples will be consumed by the process. If this application is approved, then a subset of these will be shared with Dr. Gates who is submitting a separate application for the same cruse.

(e) Will the organisms be kept alive after collection? ☐ yes ☑ no

- Specific site/location _____
- Is it an open or closed system? ☐ open ☐ closed
- Is there an outfall? ☐ yes ☐ no
- Will these organisms be housed with other organisms? If so, what are the other organisms?

(Please attach additional documentation as needed to complete the questions listed below)

9. Purpose/Need/Scope:

- To fully characterize the genetic relatedness among all individuals of two species of coral on a reef. This information is critical to understand individual variation in disease and bleaching susceptibility.

Describe how your proposed activities will help provide information or resources to fulfill the State Marine Refuge purpose and to reach the Refuge goals and objectives.

- By knowing the genotypes of all individuals on a reef and their current and future disease/ bleaching rates, we can provide managers with a predictive tool for monitoring health and differentiating between sensitive and robust sites when making protection decisions. This information is reef and site specific.

- Describe context of this activity, include history of the science for these questions and background.

See attached 9 - Context

- Explain the need for this activity and how it will help to enhance survival or recovery of refuge wildlife and habitats.

See attached 9 - Need

- Describe how your proposed project can help to better manage the State Marine Refuge.

See attached Item I9 - Management

10. Procedures (include equipment/materials)

See attached Item 10

11. Funding sources (attach copies budget & funding sources).

NSF SGER and HICRI proposal submitted and under review and HIMB-NWHI Coral Reef Research Partnership #NMSP MOA 2005-008/66882

12. List all literature cited in this application as well as all other publications relevant to the proposed project.

See attached 12

13. What types of insurance do you have in place? (attach documentation)

☐ Wreck Removal
☐ Pollution

NOAA ship Hi'ialakai is US Government owned and self insured by US government - see attached 13

14. What certifications/inspections do you have scheduled for your vessel? (attach documentation)

☑ Rat free ☐ tender vessel ☐ gear/equipment
☑ Hull inspection ☑ ballast water

See attached 14

15. Other permits (list and attach documentation of all other required Federal or State permits).

Fish and Wildlife permit being submitted simultaneously with this application.

16. Project's relationship to other research projects within the NWHI State Marine Refuge, National Wildlife Refuge, NWHI Coral Reef Ecosystem Reserve, or elsewhere.

This project is part of the National Marine Sanctuary program and HIMB Reserve partnership, HIMB-NWHI Coral Reef Research Partnership #NMSP MOA 2005-008/66882

Section C: Logistics

17. Time Frame:

Project Start Date	Project Completion Date
05/01/2006	04/30/2008

Dates actively inside the State Marine Refuge.

18 May 2006 to 14 June 2006

Personnel schedule in the State Marine Refuge (describe who will be where and when).

N/A

18. Gear and Materials

- [x] Dive equipment
- [ ] Radio Isotopes
- [x] Collecting Equipment
- [x] Chemicals (specify types)

Ethyl alcohol - see attached MSDS

19. Fixed installations and instrumentation.

- [x] Transect markers
- [ ] Acoustic receivers
- [ ] Other (specify)

20. Provide a time line for sample analysis, data analysis, write-up and publication of information.

Individuals genotyped - January 2007; data analyzed and written for publication - June 2007

21. **Vessel Information**:

Vessel Name HI`IALAKAI
IMO Number 8835619
Vessel Owner US Dep. Com. NOAA
Flag USA
Captain's Name CDR Scott Kuester
Chief Scientist or Project Leader Randall Kosaki, NOAA
Vessel Type Ocean. Res.
Call sign WTEY
Length 224'
Gross tonnage 1,914
Port of Embarkation Honolulu, HI
Last port vessel will have been at prior to this embarkation Pango Pango, Am. Samoa
Total Ballast Water Capacity: Volume 487 m3
Total number of tanks on ship 10
Total Fuel Capacity: 228,642 @ 98%
Total number of fuel tanks on ship 15

Other fuel/chemicals to be carried on board and amounts:

See attached 21

Number of tenders/skiffs aboard and specific type of motors:

See attached 21

Does the vessel have the capability to hold sewage and grey-water? Describe in detail.

See attached 21

Does the vessel have a night-time light protocol for use in the NWHI? Describe in detail (attach additional pages as necessary)

See attached 21

On what workboats (tenders) will personnel, gear and materials be transported within the State Marine Refuge?

See attached 21

How will personnel, gear and materials be transported between ship and shore?

See attached 21

If applicable, how will personnel be transported between islands within any one atoll?

See attached 21

9 – Purpose/need/scope - Context

Both natural and anthropogenic forces impact coral reefs (e.g., hurricanes and pollution). Approximately 27% of the world's coral reefs already are essentially gone and will be followed by an additional 32% that currently are considered threatened (Wilkinson 2000). If these trends are not abated, less than half of the coral reefs globally are likely to survive the next 26 years (NOAA 2003). The ecological health of any ecosystem is dependent on the persistence of adults and successful recruitment. Coral colonies are the foundation of reefs and likely are a linchpin in the health and persistence of reef systems. Although some information is available on the ecology and population connectivity of coral reefs, a clear consensus understanding of fundamental processes has not emerged. I propose to assess coral reefs at the micro spatial-scale and integrate physical and genetic data in a GIS format. The principle activity is to genotype every individual of two species of coral on a patch reef to assess relatedness at a micro-spatial scale. By combining these data in a GIS format, I hope to begin to elucidate the underlying cause of individual-to-individual differences in bleaching, disease, and survivability.

French Frigate Shoals is an ideal place because it has a broad bay, accessible coral reefs, and is minimally impacted by humans. A similar study will be conducted in Kaneohe Bay, Oahu to provide a contrast to French Frigate Shoals so that we can determine how generally applicable are our results.

9 – Purpose/need/scope - Need

Coral reefs make up only a small fraction of the worlds surface (~284,300 km^2; Spalding et al. 2001) but nonetheless are tremendously important economically and biologically. In Hawaii, utilization of coastal ecosystems result in ~$364 million dollars to the local economy annually (Cesar and van Beukering 2004) and each year in Florida coastal use contributes ~2.7 billion (Johns et al. 2001). Coral reefs have been referred to as rainforests of the sea because they support a highly diverse and interdependent ecosystem and are disappearing at an alarming rate.

The basic ecology of coral reefs and corresponding strategies for their preservation are an enormous scientific priority. Fundamental to these efforts have been recent genetic studies to determining the degree of connectivity among reefs; are coral reefs open or closed systems? Understanding the scale of dispersal for reef organisms is a necessary prerequisite to formulating marine reserve boundaries as either protecting locally self-propagating populations or as encompassing larger-scale areas that rely on regionally separated larval pools. Even for species with long larval stages, the actual degree of long-distance dispersal has been the subject of considerable debate (Roberts 1997; Jones et al. 1999; Swearer et al. 1999; Cowen et al. 2000; Rocha et al. 2002; Palumbi 2003; Taylor and Hellberg 2003). Evidence of local larval retention has been reported for a number of species with pelagic larvae, contradicting the paradigm of long distance dispersal (Hamm and Burton 2000, Taylor and Hellberg 2004).

Sedentary as adults, corals rely on free-floating larvae and gametes for dispersal and recruitment. In Pacific coral species, genetic studies based on allozymes revealed differences in the extent of gene flow between brooding and broadcast-spawning species, albeit not uniformly (Hellberg 1996; Ayre and Hughes 2000). The study by Ayre and Hughes (2000) is emblematic of the oxymoronic pattern of dispersal commonly found. These researchers assayed genetic subdivision in four broadcast spawning and four brooding species of corals along the Great Barrier Reef in Australia. The results indicated strong subdivisions within local reefs (less than

10 Km of separation) and weak to no differences between reefs separated by 500 – 1200 Km. Depending on the species, the estimated average number of individuals (N_Em) exchanged *between* reefs was 2 – 20 times *larger* than the average number exchanged between sights *within* a reef. The authors conclude that long-distance dispersal must be important over evolutionary time scales but contributes little to local recruitment. This enigmatic pattern of long-distance dispersal and limited local recruitment is common in corals. *Acropora nasuta* exhibits significant distance-dependent genetic subdivision on the Great Barrier Reef, Australia based on analyses of three nuclear introns (Mackenzie et al. 2004). In contrast, allozyme loci indicate panmixia in the closely related species, *A. valida* (Ayre and Hughes 2000). Likewise, inconsistent population subdivisions within a single mass-spawning species (*Plesiastrea versipora*) were inferred from ribosomal DNA (Rodriguez-Lanetty and Hoegh-Guldberg 2002). In this study, highly restricted gene flow was suggested among some populations (i.e., along the south-east Australian coast), whereas others appeared to be largely genetically homogeneous over similar ranges (i.e., the Ryukyu Archipelago of Japan). Regardless of the type of marker employed, patterns and mechanisms of dispersal and population interconnectivity are not well understood in corals.

What is clear is that spatial scale is a critical parameter. Nearly all studies of the genetic architecture of coral have focused on broad geographic scales, bypassing the essential issue of heterogeneity on micro-geographic scales. In essence, the field of coral population genetics has tried to move directly to macro-geographic scales and the big issues of dispersal and colonization, before conducting the essential groundwork of identifying individuals in local populations. There is a spatially explicit genetic relationship among all individual coral colonies in a coral reef, but the cause and effect of this micro-spatial structuring have rarely been considered. Dispersal at broader scales is likely to be greatly influenced by local scale heterogeneity. Therefore, understanding micro-spatial heterogeneity is critical to interpreting higher-level processes. The proposed research is intensive, but it will be the first attempt to gain a detailed picture of spatial-genetic patterning on a coral reef. It is a necessary foundation for an accurate understanding coral populations.

Objectives

I propose a 24-month study to:

- Map all individuals of *Porites lobata* and *Montipora capitata* by intensive sampling a patch reef at French Frigate Shoals. *Acropora cytherea* samples will be mapped and sampled from a section of a larger reef where disease has previously been sited.
  - The coordinates of each individual will be recorded in a GIS database, using a Global Positioning System Intelligent Buoy system.
  - All individuals and the surrounding area will be photographed to be included in the GIS database, using high-resolution digital photography,
- Genotype all individuals of both species on the reef.
  - Small samples (less than 3 cm^2) of each individual will be collected during the mapping effort to be genotyped at a minimum of 10 microsatellite loci.

Approach

- Development of genetic tools

In order to identify all individuals on the reef (i.e., genets and ramets), we are developing a microsatellite-enriched library for both species following procedures that have been successful for corals in my laboratory (Severance et al. 2004). Primers to a minimum of 10, unlinked microsatellite loci per species will be developed and tested on a sample of individuals (~15), to confirm reliability and variability. The few previous genetic studies of corals using microsatellite loci have found a significant amount of per locus variation. Our study on *Montastrea* spp. (Severance and Karl submitted) in the Caribbean found an average of 21 alleles per locus and an average heterozygosity of 63%; more then enough variation to accurately genetically identify individuals.

Coral samples will be collected by carefully removing a less than 3-cm^2 piece either at the base or edge of the colony, whichever causes the least damage. After collecting, samples will be stored in 95% EtOH. DNA will be extracted following standard procedures (Severance et al. 2004) in the laboratory at HIMB

- Surveying and sampling

We will near-saturation survey and sample *Montipora capitata* and *Porites lobata* on a small patch reef. *Acropora cytherea* will be mapped and sampled in a segment of the reef previously mapped by G. Aeby. Initial sampling will target clearly separated individual colonies and 1-meter sampling of broadly encrusting individuals. Sampling is expected to amount to ~300-500 specimens of each species.

The Intelligent GPS Buoy System (GBS) consists of four buoys with underwater acoustic receivers connected to above water GPS units. The divers carry a small, depth-recording acoustic transmitter with which the receivers can triangulate a position. Although the system has demonstrated sub-meter accuracy (~20 cm), each coral colony also will be photographed and referenced relative to a permanent markers installed on the reef. Colonies of *Porites lobata* will be sexed with histology. Small (~3cm^2) fragments of each colony will be sampled near the base of the colony to minimize damage.

The French Frigate shoals is an ideal spot because it is coral rich, accessible and provides a reference point to compare similar surveys in the more heavily impacted Main Hawaiian Islands. If this study were only done in the Main Hawaiian Islands, then the assessment of reef health would potentially be biased toward unhealthy reefs given the historical and current degradation that is happening. FFS can provide the necessary "zero point" for assessment to all other reefs in the Hawaiian archipelagos. It is also important because it is the site of some of the first observations of disease in the Northwest Hawaiian Islands. A genetic map of the region can be very informative in understanding any progression of disease.

9 – Purpose/need/scope – Management

Globally, the health of the world's coral reefs has been declining for the past couple of decades. Although sexual reproduction can be a lesser component to recruitment in many coral species, as populations become extirpated larval dispersal and successful recruitment become tremendously important. The degree of interconnectivity of coral reefs will determine how quickly, and even if, destroyed reefs will be recolonized. Previous studies have assessed the degree to which coral reef are interconnected over tens to thousands of kilometers.

Unfortunately, in many cases, the results of these efforts have proved enigmatic providing unsatisfactory and highly incomplete conclusions. The keys to understanding the interconnectedness of coral reefs and the explanation for high individual-to-individual variability in susceptibility to disease and bleaching lie in the pattern of genetic relatedness at the micro-spatial scale.

The results of this study are aimed at allowing managers to best predict which reefs as well as which individuals on a reef are most sensitive to disease and bleaching. Reefs composed of a high percentage of sensitive individuals can be identified and monitored more closely. If mitigation efforts are ever needed, understanding the correlation between genotype and health will be fundamental in choosing individuals for transplantation.

10 - Procedures

Divers will set up temporary transect makers at each side of a small patch reef. All colonies of *Montipora capitata* and *Porites lobata* along the transect line will located using underwater GPS system, photographed with size standard, and sampled by removing a small (less than 3 cm^2) piece near the base or edge of the colony. Great care will be taken to minimally impact the individual. For A. *cytherea*, mapping will be similarly done but a small branchlet will be removed from each individual. Samples will be placed in numbered bags. At the end of the dive, samples will be transferred to numbered vials and preserved with 95% Ethyl alcohol. Samples will be stored at room temperature on the ship. Samples will be transported at the end of the cruse to the Hawaii Institute of Marine Biology. DNA will be extracted from each sample using commercially available kits. DNA will be used in polymerase chain reaction amplification of 10 highly variable simple sequence loci. These loci are capable of identifying all genets uniquely.

Dr. Karl has had nearly 30 years experience SCUBA diving much of which involved collecting marine organisms. He is an Associate Researcher with nearly 20 years experience conducting molecular genetic studies. This is a new project and will make up the bulk of a Ph.D. students dissertation research, however, this student has not yet been identified.

12 - Literature

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13 - What types of insurance do you have in place?

NOAA Ship HI`IALAKAI is a U.S. Government-owned and –operated research vessel and is self-insured by the U.S. Government.

14 - What certifications/inspections do you have scheduled for your vessel?

- Rat Free (scheduled with U.S. Dept. of Health and Human Services for April 2006)
- Hull Inspection (scheduled with Hawaii Institute of Marine Biology biolobigsts (normally Scott Godwin) prior to projects working in the Northwestern Hawaiian Islands (NWHI)) to ensure no nuisance algae or other fouling species are transported to the NWHI.
- Ballast water information is transmitted to USCG as required by CFR Title 33, Vol. 2, Parts 151.1500 to 199; IMO Resolution A.868(20); and USCG COMDTPUB P16700.4

21 - Vessel Information

Other fuel/chemicals to be carried on board and amounts: gasoline – as much as 700 U.S. gal.; lube oil – as much as 10,442 U.S. gal.; numerous other industrial and household chemicals used to operate a 224-foot research vessel

Number of tenders/skiffs aboard and specific type of motors:

Ship's own tenders - 1 each 10 m AMBAR Marine jet boat with Yanmar 370-hp, Diesel inboard engine
1 each 8 m AMBAR Marine jet boat with Yanmar 315-hp, Diesel inboard engine
2 each 17.5 ft Zodiac inflatable boats, each with one Honda 50-hp, 4-stroke, outboard gasoline engine
1 each 19 ft AMBAR Marine rescue boat with Honda 115-hp, 4-stroke, outboard gasoline engine

Program-provided tenders – 19' Boston Whaler with 135 hp Honda four-stroke outboard

Does the vessel have the capability to hold sewage and grey-water? Describe in detail. The ship has a 4,000 U.S. gal Collection Holding Tank for sewage and grey water. In those waters where effluent may NOT be discharged, sewage and grey water are held in this tank until the ship is in waters where sewage and grey water may be discharged. The ship has a U.S. Coast Guard-approved Marine Sanitation Device (Omnipure model MSD 12 MC) which is used to treat sewage and grey water in those waters where effluent may be discharged.

Does the vessel have a night-time light protocol for use in the NWHI? Describe in detail. Navigation lights are on 24-hours/day. Work lights are put on at night only when conducting CTD operations. Weather decks are not illuminated at night.

On what workboats (tenders) will personnel, gear and materials be transported within the State Marine Refuge? - Personnel, gear and materials may be transported within the State Marine Refuge by the ship or any of the 5 ship's small boats listed above or by the program-provided small boat listed above.

How will personnel, gear and materials be transported between ship and shore? – Personnel, gear and materials may be transported between ship and shore by any of the 5 ship's small boats listed above or by the program-provided small boat listed above.

If applicable, how will personnel be transported between islands within any one atoll? - Personnel may be transported between islands within any one atoll by any of the 5 ship's small boats listed above or by the program-provided small boat listed above.

Karl - CV

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EMPLOYMENT	08/05 – Present	Associate Research Professor, HIMB
	08/99 – 08/05	Associate Professor, University of South Florida
	12/93 – 08/99	Assistant Professor, University of South Florida
POST-GRADUATE TRAINING	04/92 - 12/93	Post-Doctoral Fellow, Rutgers University Center for Theoretical & Applied Genetics New Brunswick, New Jersey Advisor: Robert Vrijenhoek
GRADUATE TRAINING	09/87 - 04/92	Ph. D. Genetics, awarded March 1992 University of Georgia, Athens, GA Dissertation Advisor: John C. Avise
	07/88 - 04/92	NIH Training Grant in Genetics University of Georgia, Athens, GA
	07/87 - 07/88	Teaching Assistant, General Biology University of Georgia, Athens, GA
	09/86 - 08/87	Ph. D. Genetics, Transferred University of California, Davis CA Dissertation Advisor: Francisco Ayala
	09/86 – 08/87	Teaching Assistant, General and Population Genetics University of California, Davis CA
POST BACCALAUREATE TRAINING	12/83 - 09/86	Research Assistant Hubbs-Sea World Research Institute, San Diego, CA Marine Fish Stock Assessment and Marine Mammal Demography.
	06/83 - 12/83	Field Research Assistant Marine Review Committee, San Diego, CA Ecological Studies of Near Shore Kelp Forests.
	05/82 - 06/83	Database Manager Neushul Mariculture Inc., Santa Barbara, CA Use of Near Shore Kelp Forests for Biomass Energy.

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1. Jansen, K.P. and **S.A. Karl**. Systematics of the salt marsh snakes: recent speciation and an old taxonomy. Submitted 10/2005, *Copeia*.

2. Schwartz, T.S. and **S.A. Karl**. Population genetic assignment and the origin of confiscated gopher tortoises, (Gopherus polyphemus). Submitted 1/2006 *Biological Conservation*.

3. Severance, E.G. and **S.A. Karl**. Discordant population genetic structure of sympatric mass-spawning Caribbean corals. Submitted 1/2006, *Marine Biology*.

4. Pierce, S.K., N.E. Curtis, S.E. Massey, A.L. Bass, **S.A. Karl**, and C.M. Finney. A new species of kleptoplastic sea slug from the Florida Keys. Submitted, 10/2005.

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9. Cattell, M. and **S.A. Karl**. 2004. Genetics and morphology in a Borrichia frutescens and B. arborescens (Asteraceae) hybrid zone. *American Journal of Botany* 91:1757-1766

10. Hayes, K. and **S.A. Karl**. 2004. Characterization of microsatellite markers from the gastropod genus *Melongena*. *Molecular Ecology Primer Notes* 4:755-757/

11. Roberts, M., T. Schwartz and **S.A. Karl**. 2004. Global population structure and male-mediated gene flow in the green turtle (Chelonia mydas): Microsatellite analysis. *Genetics* 166:1857-1870.

12. Seminoff, J.A., **S.A. Karl**, T. Schwartz, and A. Resendiz. 2004. Hybridization of Chelonia mydas and Eretmochelys imbricata in the Pacific Ocean: Indication of an absence of gender bias in the directionality of crosses. In press, *Bulletin of Marine Science* 73:643-652.

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14. Severance, E.G., A.M. Szmant, and **S.A. Karl.** 2004. Microsatellite loci isolated from the Caribbean coral, Montastraea annularis. *Molecular Ecology Primer Notes* 4:74-76.

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18. **Karl, S.A.** and D.S. Wilson. 2001. Phylogeography and systematics of the mud turtle, Kinosternon baurii. *Copeia* 101:797-801.

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20. **Karl, S.A**. and B.W. Bowen. 1999. Evolutionary significant units versus geopolitical taxonomy: Molecular systematics of an endangered sea turtle (genus Chelonia). *Conservation Biology* 13:990-999

21. Garzon, M., N. Jonoska, and **S.A. Karl**. 1999. The bounded complexity of DNA computing. *Biosystem* 52:63-72.

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29. Hare, M.P., **S.A. Karl** and J.C. Avise. 1996. Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. *Molecular Biology and Evolution* 13:334-345.

30. **Karl, S.A.**, S. Schutz, D. Desbruyères, R.C. Vrijenhoek and R. Lutz. 1996. Molecular analysis of gene flow in the hydrothermal-vent clam, Calyptogena magnifica. *Molecular Marine Biology and Biotechnology* 5:193-202.

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33. **Karl, S.A.** and J.C. Avise. 1993. PCR-based assays of polymorphisms from anonymous single-copy nuclear DNA: Techniques and applications. *Molecular Biology and Evolution* 10:342-361.

34. **Karl, S.A.**, B.W. Bowen, and J.C. Avise. 1992. Global population structure and male-mediated gene flow in the green turtle (Chelonia mydas): RFLP analysis of anonymous nuclear DNA regions. *Genetics* 131:163-173.

35. **Karl, S.A.** and J.C. Avise. 1992. Balancing selection at allozyme loci in oysters: Implications from nuclear RFLPs. *Science* 256:100-102.

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38. Wilson, D.S. and **S.A. Karl**. 2001. Genetic relatedness of populations of the mud turtle, Kinosternon baurii, from mainland Florida and the upper and lower Florida Keys. *Chelonia Conservation and Biology* 4:228-230

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PUBLICATIONS IN PREPARATION

45. Dewar, H., T. Thys, J. O'Sullivan, C. Farwell, A. Wallis, T. Tobayama, Y. Kondo, J.T. Streelman, A.L. Bass, C. Puchulutegui & **S.A. Karl**. Basking and diving in Japanese waters: movements and behaviors of the ocean sunfish (Mola mola). In revision, *Marine Biology*.

46. Severance, E.G. and **S.A. Karl.** Reconstructing gene flow and evolution in the Caribbean coral, Montastraea faveolata. In preparation, *Conservation Genetics*.

47. Curtis, C., B.S. Stewart, and **S.A. Karl**. Molecular identification of the sex of pinnipeds. In preparation, *Conservation Genetics*.

48. **Karl, S.A.**, C. Curtis, and M. Cattell. Molecular phylogenetics and evolution of the surfperch family (Embiotocidae). In preparation, *Molecular Phylogenetics and Evolution.*

49. Jansen, K.J. and **S.A. Karl**. Population genetics of the mangrove salt marsh snake, Nerodia clarkii compressicauda, in a fragmented, linear habitat. In preparation, *Conservation Genetics*.

50. Hayes, K. and **S.A. Karl**. Mitochondrial phylogeny of crown conchs: The Corona complex simplified. In preparation, *Molecular Phylogenetics and Evolution.*

PRESENTATIONS (presenter listed first)

Invited

1. **Karl, S. A.** 2004. Complex population structure in turtles. Workshop on the Use of molecular markers for the study of marine biodiversity. Horta, Azores. July 2004

2. **Karl, S. A.** 2002. A talk in two parts: Evolution of larval life history in a Polychaete worm, and genetics and the ocean sunfish, Mola mola: Millstone or milestone?" Presented to the University of South Carolina, Marine Science Program. Columbia, SC, USA, 5 April.

3. **Karl, S. A.,** A.L. Bass, M.H. Zacks. 2000. Marine turtles and international considerations for listing ESUs. Presented to the Society for Conservation Biology. Missoula, MT, USA, 8-12 June.

4. Zacks, M.H. and **S.A. Karl.** 2000. [De]constructing archetypes of the pristine. Presented to the Latin American Studies Association, 12th International Congress. Miami, FL, USA, 16-18 March.

5. **Karl, S.A.** 2000. Evolutionary genetics of Chelonia. Special Symposium at the XX Presented to the International Symposium on Turtle Biology, Orlando, FL, USA, 29 February - 4 March.

6. **Karl, S.A.** 1999. Living by the numbers: The commingling of mathematics and biology. Keynote Address. Presented to the 24th Annual Sun Coast Regional Meeting of the Florida Section of the Mathematical Association of America. Tampa, FL, USA, 3 December.

7. **Karl, S.A.** 1999. Blending the alphabet soups: PCR meets ESUs. Presented to A Second Symposium on the Status and Conservation of Florida Turtles. St. Petersburg, FL, USA, 8-11 October.

8. **Karl, S.A.** 1999. Getting our bearings in DNA computing, Panelist. Presented to the Genetic and Evolutionary Computation Conference. Orlando, FL, USA, 13 July.

9. **Karl, S.A.** 1995. Hybridization and taxonomy of marine turtles: Anonymous nuclear DNA sequence analyses. Presented to the Sea Turtle Genetics Workshop, National Marine Fisheries Service, Miami, FL, USA, 12-14 September.

10. **Karl, S. A.** 1995. From Playa Grande to the stygian deep-sea: Gene flow in the marine environment. Presented to the Florida International University Graduate Seminar Series. Miami, FL, USA, 19 September.

11. **Karl, S.A.** 1994. Genetic variation at anonymous nuclear DNA loci. Presented to the American Association for the Advancement of Science, Molecular Conservation Genetics Symposium. San Francisco, CA, USA, 19-22 June.

12. **Karl, S.A.** 1994. Nuclear DNA analysis of the green turtle (Chelonia mydas). Presented to the University of Florida, BEECS Genetics Core, Conservation Genetics Symposium. Gainesville, FL, USA, 21-26 February.

13. **Karl, S.A.** 1994. Dispersal of hydrothermal vent organisms in the stygian deep-sea. Presented to the Archbold Biological Station. Lake Placid, FL, USA, 5 May.

14. **Karl, S.A.** 1994. Genetic issues in conservation biology. Symposium on the Status and Conservation of Florida Turtles. St. Petersburg, FL, USA, 2-3 April.

15. **Karl, S.A.** 1992. PCR-based assays of polymorphisms from anonymous single-copy nuclear DNA: Techniques and applications to green turtles and American oyster. Presented to the Haskins Shellfish Research Center. Bivalve, NJ, USA, 15 February.

Other

16. Hayes, K. and **S.A. Karl**. 2002. Evolution of the "Corona Complex" in the genus Melongena (Gastropoda: Melongenidae). Presented at the 16th Annual Meeting of Florida Association of Benthologists. St. Augustine, Florida. 21 November.

17. Hayes, K. and **S.A. Karl**. 2001. Evolution of the "Corona Complex" in the genus Melongena (Gastropoda: Melongenidae). Presented to the 14th International Congress of Unitas Malacologica and the 67th Annual Meeting of the American Malacological Society. Vienna, Austria. 19-25. August.

18. Schwartz, T. and S.A. Karl. 2001. Population genetics of the gopher tortoise (Gopherus polyphemus) in Florida. Presented to the Annual Society for Integrated and Comparative Biology. Chicago, IL, USA, 3-7 January.

19. Schwartz, T. and **S.A. Karl**. 2000. Genetic Structure of the gopher tortoise (Gopherus polyphemus) populations in Florida using microsatellites. Poster presented to the Annual Suncoast Biomolecular Symposium. Tampa, FL, USA, 20 October.

20. Hayes, K. and **S.A. Karl**. 2000. Evolution of the "Corona Complex" in the genus Melongena (gastropoda: melongenidae): Patterns from internal transcribed spacer regions and microsatellite data. Poster presented to the Annual Suncoast Biomolecular Symposium. Tampa, FL, USA, 20 October.

21. Severance, E. and **S.A. Karl**. 2000. Molecular evolution of Scleractinian diversity: How panmictic are coral populations of the Western Atlantic? Presented to the Annual Meeting of the Society for the Study of Evolution, Bloomington. ID, USA, 23-27 June.

22. Schwartz, T., **S.A. Karl**, H. Mushinsky, and E. McCoy. 2000. Genetic Structure of the gopher tortoise (Gopherus polyphemus) populations in Florida using microsatellites. Poster presented to the Annual Meeting of the Society for the Study of Evolution. Bloomington, ID, USA, 23-27 June.

23. Hayes, K. and **S.A. Karl**. 2000. Evolution of the "Corona Complex" in the genus Melongena (Gastropoda: Melongenidae): Evidence from internal transcribed spacer regions and microsatellite data. Poster presented to the Annual Meeting of the Society for the Study of Evolution. Bloomington, ID, USA, 23-27 June.

24. Streelman, J.T., M.E. Alfaro, M.W. Westneat, and **S.A. Karl**. 1999. Molecular phylogeny and evolution of parrotfishes. Presented to the Annual Meeting of the Society for the Study of Evolution. Madison, WI, USA, 22-26 June.

25. Schultze, S.R, **S.A. Karl**, S.A. Rice, and J.L. Simon. 1999. Biogeography and evolution of poecilogony in the polychaete genus Streblospio: Evidence from molecular genetics and reproductive compatibility trials. Presented to the Annual Meeting of the Society for the Study of Evolution. Madison, WI, USA, 22-26 June.

26. Severance, E.G. and **S.A. Karl**. 1999. Estimating molecular biodiversity through gene flow analysis of coral from the Florida Keys. Presented to the International conference on the Scientific Aspects of Coral Reef Assessment, Monitoring, and Restoration. Ft. Lauderdale, FL, USA, 14-16 April.

27. Jansen, K.P., **S.A. Karl**, and H. Mushinsky. 1998. Molecular systematics and phylogeography of the salt-marsh snakes, Nerodia clarkii, using mtDNA d-loop sequences. Presented to the 78th Annual Meeting of the American Society of Ichthyologists and Herpetologists. University of Guelph, Guelph, Canada, 16-22 July.

28. **Karl, S.A.** and J.T. Streelman. 1998. Are microsatellite loci really neutral genetic markers? Presented to the Annual Meeting of the Society for the Study of Evolution. Vancouver, Canada, 10-24 June.

29. Streelman, J.T. and **Karl, S.A.** 1998. Timing the divergence of cichlid major groups. Presented to the Annual Meeting of the Society for the Study of Evolution. Vancouver, Canada, 10-24 June.

30. Schulze, S.R., **S.A. Karl**, J.L. Simon, S.A. Rice. 1998. Reproductive compatibility and genetic relatedness of the polychaete Streblospio benedicti species complex. Presented to the 27th Annual Benthic Ecology Meetings. Melbourne, FL, USA, 13-15 March.

31. Bowen, B.W. and **S.A. Karl**. 1998. The gods of conservation biology. Presented to the XVIII International Symposium on Turtle Biology. La Paz, Mexico, 2-7 March.

32. **Karl, S.A.**, D. Sweger, and E. Severance. 1997. Molecular systematics of marine turtles using single-copy nuclear DNA sequence data. Presented to the 77th annual meeting of the American Society of Ichthyologists and Herpetologists. University of Washington, Seattle, WA, USA, 26 June-2 July.

33. Streelman, J.T., and **S.A. Karl**. 1997. Nuclear DNA phylogeny and evolution of labroid fishes. Presented to the 77th annual meeting of the American Society of Ichthyologists and Herpetologists. University of Washington, Seattle, WA, USA, 26 June-2 July.

34. **Karl, S.A.** 1997. Taxonomic distinctiveness of the black turtle, Chelonia agassizii: From green to black and back. Presented to the XVII International Symposium on Turtle Biology. Orlando, FL, USA, 4-8 March.

35. Streelman, J.T., and **S.A. Karl**. 1996. Evolution of nuclear DNA in cichlid fishes. Presented to the 76th annual meeting of the American Society of Ichthyologists and Herpetologists. New Orleans, LA, USA, 13-19 June.

36. Jonoska, N. and **S.A. Karl**. 1996. A molecular computation of the road-coloring problem. Presented to the 2nd DIMACS annual workshop on DNA Based Computer. Princeton, NJ, USA, 10-12 June (all authors presented).

37. **Karl, S.A.** 1994. Genetic issues in conservation biology. Presented to the Symposium on the Status and Conservation of Florida Turtles. St. Petersburg, FL, USA, 2-3 April.

38. **Karl, S.A.**, S. Schutz and R.C. Vrijenhoek. 1993. Population genetics and gene flow in the deep-sea-hydrothermal vent clam Calyptogena magnifica. Presented to the Society for Molecular Biology and Evolution. Irvine, CA, USA, 7-10 July.

39. **Karl, S.A.** 1993. Population genetics and gene flow in the deep-sea-hydrothermal vent clam Calyptogena magnifica. Presented to the Society for the Study of Evolution. Snowbird, UT, USA, 10-14 June.

40. **Karl, S.A.**, B.W. Bowen and J.C. Avise. 1992. Global population structure and male-mediated gene flow in the green turtle (Chelonia mydas): RFLP Analyses of anonymous nuclear DNA regions. Presented to the XII International Symposium on Turtle Biology. Jekyll Island, SC, USA, 24-29 February.

41. **Bowen, B.W.**, S.A. Karl and M. Marcovaldi. 1992. Molecular confirmation of marine turtle hybrids. Poster presented to the XII International Symposium on Turtle Biology. Jekyll Island, SC, USA, 24-29 February.

42. **Karl, S.A.**, B.W. Bowen and J.C. Avise. 1991. Global population structure and male-mediated gene flow in the green turtle (Chelonia mydas): RFLP Analyses of anonymous nuclear DNA regions. Presented to the Society for the Study of Evolution. Hilo, Hawaii, USA, 28 July - 3 August.

43. **Karl, S.A.**, B.W. Bowen and J.C. Avise. 1990. Genetic analysis of the green turtle, Chelonia mydas. Presented to the Tenth Annual Workshop on Sea Turtle Conservation and Biology. Hilton Head Island, South Carolina, USA, 20-24 February.

44. **Karl, S.A.** 1987. Genetic analysis of the chukar partridge (Alectoris chukar) from San Nicolas Island, CA. Presented to the Channel Island Symposium. Santa Barbara CA, USA, 2-6 March.

GRANTS AWARDED

1. 01/05 to 12/05 — Arcadia Wildlife Preserve, Inc. PI: **S.A. Karl**. "Conservation of an open ocean giant: telemetry and genetics of the ocean sunfish (Molidae)." $6,000

2. 10/04 to 09/06 — International Game Fish Association. PI: **S.A. Karl**. "Resolving Evolutionary Lineages of Bonefish (*Albula spp.*)." $9,000.

3. 08/04 to 11/04 Florida Fish and Wildlife Conservation Corp. PI: **S.A. Karl**. "Consultation on genetics of Dinoflagellates for ECOHAB." $21,000.

4. 03/04 to 02/05. Arcadia Wildlife Preserve, Inc. PI: **S.A. Karl**. "Conservation of an open ocean giant: telemetry and genetics of the ocean sunfish (Molidae)." $2,000.

5. 06/03 to 05/05. National Science Foundation, Systematic and Population Biology. PI: **S.A. Karl**, CoPI (RUI): S. Rice. "Collaborative Research: Molecular phylogenetics of the globally distributed polychaete, Polydora cornuta." est. $100,000.

6. 06/03 to 05/04 Arcadia Wildlife Preserve, Inc. PI: **S.A. Karl**, CoPI: T. Schwartz "Population genetics of the gopher tortoise (Gopherus polyphemus) in Florida." $5,000.

7. 01/03 to 07/04. National Geographic Society. PI: T. Thys, CoPIs: H. Dewar, **S.A. Karl**, and J.T. Streelman. "Conserving an open ocean giant: telemetry and genetics of Mola mola." $25,000.

8. 04/02 to 05/02 Florida Institute of Oceanography. Suncoaster ship time grant. PI: **S.A. Karl**. "Molecular ecology and evolution of coral and coral reef fish." $16,000.

9. 08/01 to 07/03 Florida Fish and Wildlife Service. PI: **S.A. Karl**. "Genetic analysis of Cape Sable seaside sparrow populations." $57,120.

10. 01/01 to 02/01 Florida Institute of Oceanography. Bellows ship time grant. PI: **S.A. Karl**. "Molecular ecology and evolution of coral and coral reef fish." $14,000.

11. 08/00 to 07/03 Arcadia Wildlife Preserve, Inc. PI: S.A. Karl, CoPI: T. Schwartz. "Population genetics of the gopher tortoise (Gopherus polyphemus) in Florida." $4,200.

12. 05/00 to 06/02 National Geographic Society. PI: H. Dewar, CoPI: **S.A. Karl**, J.T. Streelman, and T. Thys. "Conserving an open-ocean giant: telemetry and genetics of Mola mola." $30,000.

13. 01/00 to 02/00 Florida Institute of Oceanography. Bellows ship time grant. PI: **S.A. Karl**. "Molecular ecology and evolution of coral and coral reef fish." $14,000.

14. 06/99 - 05/00 Department of Environmental Protection. PI: B. W. Bowen, CoPI: **S.A. Karl**. "Systematics and ecology of bonefish (Albula spp.) in Florida waters." $76,880.

15. 08/98 - 07/01 National Science Foundation, Systematic and Population Biology. PI: **S.A. Karl**. "Molecular phylogeny and evolution of labroid fishes." $180,000.

16. 02/97 - 01/01 Florida Game and Fresh Water Fish Commission, PI: **S.A. Karl**, CoPI: K. P. Jansen and H. R. Mushinsky. "Ecological genetics of the saltmarsh snake, Nerodia clarkii." $5,600.

17. 04/95 - 03/96 USF Research and Creative Scholarship, PI: **S.A. Karl**. "Fine-scale molecular genetic analysis of the deep-sea hydrothermal vent tube worm, Riftia pachyptilia." $7,500.

18. 03/92 - 06/95 National Science Foundation, Dissertation Improvement Grant. PI: **S.A. Karl.** "Single copy nuclear DNA genetic assessment of natural populations." $6,750 (declined due to graduation).

19. 09/92 09/94 National Science Foundation, Post Doctoral Marine Biotech Fellowship, PI: **S.A. Karl**, CoPI: R. Vrijenhoek and R. Lutz. "Population genetics and gene flow in hydrothermal vent communities: Molecular assessment of genetic variation." $86,000.

PAST POSTDOCTORAL ADVISEES

Dr. Clark Craddock, 7/1/95 to 6/30/96

CURRENT GRADUATE STUDENTS (*co-chair of committee)

Ph. D.

1. A. Bass: Systematics, Sea slugs
2. A. Castro: Pop. Genetics, Sharks
3. C. Curtis: Pop. Genetics, Seals

M. S.

PAST GRADUATE STUDENT ADVISEES (graduated: F=Fall, S=Spring, Sm=Summer)

Ph. D.

1. M. Cattell*; S01: Plant Hybridization
2. A. Jackson: Pop. Genetics, Shrimp
3. K. Jansen*; S01: Mol. Conserv., Snakes
4. E. Severance: Phylogeography, Coral
4. JT. Streelman*§; F98: Systematics, Fish
5. M. Tringalli: Pop. Genetics, Fish

M. S.

5. K. Hayes: Phylogeography, Snails
6. M. Roberts; F98: Genetics, Sea Turtles
7. S. Schulze*; S99: Genetics, Polycheates
8. T. Schwartz: Mol. Conserv., Tortoises
9. M. Stone; S99: Invert. Community Ecol.

*co-chair of committee §awarded a NSF/Sloan Postdoctoral Fellowship.

CURRENT GRADUATE STUDENT COMMITTEES

Ph. D.

1. T. Campbel (USF)

M. S.

2. S. Hosain (USF)

PAST GRADUATE STUDENT COMMITTEES (graduated: F=Fall, S=Spring, Sm=Summer)

Ph. D.

3. P. Delis (USF); S01
4. K. Donaldson (Dept. Mar. Sci.); S01
5. O. Hernandez (USF); S98
6. A. Matos (USF); S00
7. B. Warwick (Dept. Mar. Sci.)
8. K. McCarthy (FL Int. Uni.)
9. L. Rocha (Univ. FL)

M. S.

10. C. Crawford (Dept. Mar. Sci.); S01
11. L. S. Cho Chumg Hing (USF); S95
12. L. Dryden (USF); S97
13. J. Kirsten (USF); F96
14. A. Morales (USF); S95
15. C. Steffen (USF); Sm98
16. B. Warwick (USF); S99
17. J. Colson (USF)
18. H. Hamilton (USF)

UNDERGRADUATE HONORS THESIS COMMITTEES

Heather Stevens; S95
Amanda Ellerd; S99

UNDERGRADUATE ADVISEES

1. A. Balar; Sm95
2. B. Boss; F94, Sm94
3. R. Brachmann; S01, Sm01
4. M. Evans; Sm97
5. S. Jones; F97, S98
6. R. Juemmankhan; Sm95
7. A. Ledger; Sm99, F99, S00
8. S. Lutz; Sm96, F96, S96
9. M. Moody; S02, Sm02, F02
10. S. Nyland; F94
11. F. Pirone; F94
12. E. Ponty; S96
13. C. Puchulutegui; S00 - S02
14. J. Rifkin; Sm97
15. M. Stabinski; S95
16. M. Smith; F00, S01, Sm01
17. D. Sweger; F94, Sm94
18. B. Wawrik; Sm96
19. V. Rusin-West; S98
20. J. Wittkopp; Sm96, F96
21. C. M. Zajac; Sm95

PROFESSIONAL SERVICE

Associate Editor for Evolution, 1 January 2000 – 31 December 2003.
Reviewer for numerous manuscripts and grants from national and international Journals and organizations.
National Science Foundation, Population Biology Grant Panel, 17 – 19 October 2001.
National Science Foundation, National Ecological Observatory Network Workshop, 10 - 11 March 2000.
National Science Foundation Dissertation Improvement Grant Panelist, February 1998.

PROFESSIONAL SOCIETY AFFILIATIONS

American Association for the Advancement of Science.
Genetics Society of America
Sigma Xi.
Society for the Study of Evolution.